18

Beneficial biofilms: wastewater and other industrial applications

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Abstract: This chapter describes the use of beneficial biofilms for the production of industrial chemicals such as ethanol, butanol, lactic acid, acetic acid/vinegar, succinic acid, and fumaric acid. It also emphasizes application of biofilm reactors for treatment of dairy industry wastewater, oily sea water, and wastewater in general. The bioreactor designs where these biofilms are used can be batch, continuous stirred tank (CSTR), packed bed (PBR), fluidized bed (FBR), airlift (ALR), or any other suitable reactor configuration. Biofilm reactors are simple, offer higher productivities, and biofilm supports such as bone char, and clay brick particles are economically available.

Key words: biofilms, industrial chemicals, ethanol, butanol, lactic acid, acetic acid/vinegar, biofilm reactors, biofilm supports.

18.1 Introduction

Biofilm formation is a natural process, where microbial cells attach to solid surfaces and grow in the form of uniform layers of cells. Common examples of biofilms include slimy and slippery films found on stones and rocks submerged in water streams and deep under the ocean. Other examples of biofilms include cell growth on hulls of ships (water submerged surfaces), inside heat exchange equipment, inside surfaces of pipes carrying liquid foods and even on teeth and medical equipment. The amount of cell growth and formation of biofilm layers depend upon availability of nutrients and favorable environmental conditions. Since biofilm formation on ship surfaces and heat exchange equipment is not desirable, removing

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them is an involved process which costs the shipping and chemical industry a significant amount of money. However, this natural process of biofilm formation either by multi or single microbial species can be exploited for obtaining economic gains. The reader is advised that biofilm formation requires no exogenous chemical or physical process as bacteria (or other microbial cells) can grow on nearly all surfaces. A question arises as why cells grow on surfaces? Attempts to answer this question have been, at least partly, reported in literature (Costerton *et al.*, 1987; O'Toole *et al.*, 2000; Qureshi *et al.*, 2005) and hence are considered beyond the scope of this article.

Bioconversion and microbial biotechnology is an environmentally friendly and rapidly growing area where chemicals and biochemicals can be produced using microbial cultures. The production of such chemicals and biochemicals involves various costs including substrate, process, and recovery. In order to reduce the overall cost of production of a chemical or biochemical, the processing cost should be reduced. One of the goals in microbial bioconversion is to increase cell concentration inside the reactors by cell immobilization or cell recycle for the purpose of obtaining high reaction rates thus reducing reactor size and process time. Reduction in reactor size and process time reduces both capital and process costs thus producing chemicals more economically. Cell immobilization generally requires expensive chemicals thus offsetting economic gains obtained by increasing cell concentration inside the reactor. Similarly cell recycle technology is cost intensive. Since increasing cell concentration inside the reactor either by immobilization or cell recycle techniques is costly, various authors have employed the natural process of adsorption and biofilm formation on solid surfaces (outlined in the first paragraph of this section) and used them in reactors for the production of chemicals cost effectively (Qureshi and Maddox, 1987; Krug and Daugulis, 1983; Tyagi and Ghose, 1982; Crueger and Crueger, 1989).

Microbial cells can adhere and multiply onto solid surfaces of natural origin and this property can be employed for increasing cell concentration inside the reactors and produce chemicals at faster rates thus reducing capital and process costs that are listed in the above paragraph. It has been observed that cell concentration in adsorbed cell reactors can be increased to 50–60 gL^{-1} (Qureshi *et al.*, 2005) as compared to 3–4 gL^{-1} in free cell batch reactors. Such an increase in cell concentration results in enhanced reaction rates. It should be noted that the process of cell adsorption results in a number of advantages including:

i) requirements for no exogenous chemicals
ii) the process of adsorption is simple and can be performed in place
iii) reactors where adsorbed cell biofilms are used are simple in concept and construction
iv) reaction rates are high

v) adsorption supports are inexpensive and are of natural origin and environmentally friendly
vi) high cell concentrations are achieved inside the reactors.

The details of the reactors that can be used for biofilms have been given in Section 18.4. The purpose of this chapter is to inform the reader that biofilms can be successfully employed for production of various chemicals and hence providing economic benefits to mankind.

18.2 Various types of biofilms

Biofilms can be categorized into two groups, namely: i) multi-species biofilms and ii) single species biofilms.

i) In multi-species bacterial communities each species fills an ecological niche within the biofilm depending on its metabolism and morphology (Stoodley *et al.*, 2002). The nature of mixed culture biofilms is dependent on which species are present and what role each species plays. For example, a single species may utilize anaerobic fermentation deep within one biofilm in one environment, but may utilize an aerobic metabolism in another environment in the presence of different neighboring biofilm species. It has been observed that multi-species biofilms are important both clinically as well as industrially. Clinically, biofilms are undesirable and important as a source of persistent infections. They are responsible for dental caries and nosocomial infections, as well as a variety of other infections and diseases (Costerton *et al.*, 1999). Industrially, biofilms are detrimental in many cases and beneficial in many others. For example, natural biofilms can reduce heat transfer in heat exchangers and cooling towers (Mortensen and Conley, 1994), foul ultrafiltration and reverse osmosis membranes (McDonogh *et al.*, 1994), and contaminate food processing equipment (Carpentier and Cerf, 1993). Beneficial uses of multi-species biofilms include the treatment of wastewater for removal of organics (Taras *et al.*, 2005; Hall, 1987) and heavy metals (Meyer and Wallis, 1997).

ii) Single species biofilms are used to produce commercially important chemicals (Qureshi and Maddox, 1990; Maddox *et al.*, 1988). Such biofilms can exist in some situations and are important industrially, although in nature they are not the norm. For chemical production, single species biofilms are important because they allow for control and maximization of desired products. In this case, a single species is inoculated into a sterile environment and allowed to form a biofilm before being used to produce a particular chemical product.

Both multi and single species biofilms can further be divided into two groups, namely: a) biofilms that grow onto supports including bonechar, charcoal, resin, concrete, clay brick, or sand particles and, b) biofilms that

are formed as a result of flocs and aggregate formation. On the above mentioned supports, biomass grows all around the particles and the size of the biofilm particles grows over time usually to several mm in diameter. The density of the support particles is usually higher than the fermentation broth and for this reason bioparticles tend to remain in the lower section of the reactor. The second type of biofilm that is mentioned above is where no support is used and cells form biomass granules and flocs that also grow in size over time. This type of biofilm is called granular biofilm and the reactor where this biofilm is used is called a granular biofilm reactor (GBR). Granule formation may take from several weeks to several months. During biofilm formation, the cells produce extracellular polymeric substances (EPS) that bind the cells firmly in the form of flocs and aggregates.

18.3 Factors enhancing biofilm formation

Several parameters determine how quickly biofilms form and mature, including the surfaces of adsorbents and bacterial species, the cellular makeup of the culture, and environmental factors (such as nutrients present in the medium, temperature, and pH). Rough surfaces and pores inside porous supports tend to enhance biofilm formation (Characklis *et al.*, 1990) as shear forces are lower near a rough surface, and there is a larger surface area to which cells can adhere and multiply. Pores provide a protected environment for cells to attach. Biofilm formation also tends to increase with the hydrophobicity of the surface material (Donlan and Costerton, 2002). Biofilms tend to form more readily in the presence of ample nutrients (Cowan *et al.*, 1991). One function of the biofilm is to anchor cells in a friendly, nutrient-rich environment. Phosphorus is a particularly important nutrient. Cells saturated with phosphate have a greater tendency to flocculate and adhere due to their increased hydrophobicity, while those cells depleted in phosphate are more hydrophilic and less likely to adhere (Bücks *et al.*, 1988). A hydrophobic cell will be more able to overcome the initial electrostatic repulsion with the solid surface and adhere more readily. The presence of fimbriae, proteinaceous bacterial appendages high in hydrophobic amino acids, can increase cell surface hydrophobicity (Donlan and Costerton, 2002; Donlan, 1992). Flagellated cells, where flagellar motility may serve to overcome initial electrostatic surface repulsion, also show increased ability to attach to surfaces.

18.4 Biofilm reactors

Biofilm reactors can be assembled in a number of configurations including batch, continuous stirred tank (CSTR; including agitating continuous reactors, and rotary continuous reactors), packed bed (PBR), trickling bed

(TBR), fluidized bed (FBR), airlift (ALR), upflow anaerobic sludge blanket (UASB), expanded granular sludge bed (EGSB), expanded bed (EBR), granular biofilm (GBR), moving bed biofilm (MBBR), and vertical moving biofilm (VMBR) reactors. The details of some of these reactors including schematic diagrams are published elsewhere (Qureshi *et al.*, 2005).

Airlift reactors contain two concentric tubes, a riser (an inner tube) and a downcomer (an outer tube). In these reactors, mixing is achieved by circulating or injecting air at the bottom of the reactor. As a result of force applied by the air (at the bottom of the inner tube), the liquid in the inner tube moves up which then overflows (the inner tube) downward thus creating eddies to mix the liquid. In some airlift reactors the downcomer is replaced with an external loop to circulate fermentation broth or medium. Such reactors where air is replaced by anaerobic gases (N_2 or CO_2 and H_2) are called gaslift reactors. UASB reactors (contain granular biofilm particles; developed by Lettinga *et al.*, 1980) are used for anaerobic treatment of wastewater/industrial effluents. As the name suggests, the flow in these reactors is in an upward direction. At the top of the reactor, provisions are made for gas(es) to escape and sludge particles to settle to the bottom part of the reactor. Reactor effluent is removed from the top of the reactor.

18.5 Biofilms in biological wastewater treatment

Many chemical, biological, and food processing industries produce large amounts of effluents that cannot be discharged into water streams as they are environmental pollutants and are toxic to marine life and humans. Hence, their treatment is sought prior to such a discharge. Use of biofilm technology is one area which has been successfully employed to treat such wastewater streams. The application of biofilm technology in wastewater treatment originated from the industrial operation of trickling filters in the early 1880s in Wales, Great Britain (Lazarova and Manem, 2000). Biofilm processes in wastewater treatment can be divided into two groups, namely: i) the fixed-medium systems where the biofilm media are static in the reactors and the biological reactions take place in the biofilm developed on the static media, and ii) the moving-medium systems where the biofilm media are kept continually moving by means of mechanical, hydraulic, or air forces (Rodgers and Zhan, 2003). The fixed-medium systems include trickling filters and biological aerated filters, while the moving-medium systems include rotating biological contactors (RBC), MBBRs, VMBRs, and FBRs. Rotating biological contactors treat wastewater streams using a thin biofilm of aerobic microorganisms on rotating cylinders or biodiscs. The rate of rotation is selected to provide optimum contact of the waste stream with the biofilm for efficient oxygen transfer and bioactivity (reviewed in Qureshi *et al.*, 2005).

Before a biofilm-based treatment system is to be considered for the treatment of wastewater, it is necessary to determine whether the naturally occurring microorganisms are able to produce biofilms, while simultaneously reducing the COD (chemical oxygen demand) of the wastewater, or if there is a need to inoculate the reactor with external bacterial strains. The most commonly used RBC is the rotating biodisc and its various modifications (Kargi and Eker, 2001). In this study, Kargi and Eker (2001) have shown that a rotating-perforated-tube biofilm reactor is effective in COD removal from synthetic wastewater composed of diluted molasses, urea, KH_2PO_4, and $MgSO_4$. The liquid phase in the tank was not aerated, (the total biofilm surface area was 1.34 m^2), and the rotation speed of the tubes was 5 rpm (Kargi and Eker, 2001).

The fluidized bed biofilm reactors (FBBR; also called FBR), in which particles move up and down within the expanded bed in the well defined zone of the reactor, have been used for more than two decades for treating industrial wastewater (Jesis *et al.*, 1977; Rabah and Dahab, 2004). Adsorbed cell bacterial systems used in FBBRs offer some technical advantages. Since chemical wastes are injected into the recycle stream (of FBBR), toxic chemicals present in feed are immediately diluted, which make the microorganisms more adapting to chemical toxicity than in many other conventional reactor systems. In addition, since FBBRs are usually oxygenated by supplying air into the recycle loop, a high level of microbial activity may be supported with minimal air stripping of volatile chemicals.

Jesis *et al.* (1977) studied FBBR, and they found that the use of small, fluidized media enabled the FBBR to retain high biomass concentrations and, thereby, operate at significantly reduced hydraulic retention times. In pilot scale operations these authors reported that when the volatile solids concentrations were between 30 000 to 40 000 mgL^{-1} during denitrification operation, 99% of influent nitrates could be removed under hydraulic retention times as low as 6 min. In a similar study, Rabah and Dahab (2004) (during their evaluation of the use of fluidized-bed biofilm reactors for nitrate removal) concluded that the FBBR system is capable of handling an exceptionally high nitrate nitrogen concentration of 1000 $mg(N)L^{-1}$ with very high removal efficiency (up to 99.8%). The efficiency of the FBBR can be up to 10 times greater than that of the activated sludge system and typically occupies only 10% of the space required by stirred tank reactors of similar capacities (Rabah and Dahab, 2004). Higher biomass concentration in the FBBRs (40 000 mgL^{-1}) compared to 3 000 mgL^{-1} in the activated sludge have been shown to be the reason for this greater efficiency (Shieh and Keenan, 1986). Anaerobic treatment of wastewater in FBBRs is another area that has been studied extensively (Iza, 1991). In this article, Iza (1991) presented the theoretical basis for design and operation of a FBR for anaerobic treatment of wastewater.

Use of UASB is another process that has been successfully applied in wastewater treatment (Seghezzo *et al.*, 1998). The development of UASB

saw a significant increase in anaerobic removal of various chemicals from the wastewater using these reactors. The examples include anaerobic removal of pentachlorophenol (Ye *et al.*, 2004), nitrogen removal (Schmidt *et al.*, 2004), dechlorination using *Dehalospirillum multivorns* (Horber *et al.*, 1998), anaerobic treatment of municipal solid leachate (Iza *et al.*, 1992), and starch degradation (Fang and Hui, 1994). More studies on this subject have been reviewed in Veeresh *et al.* (2005) (anaerobic treatment of phenol and cresols in UASB reactors). The success of the UASB concept relies on the establishment of a dense sludge bed in the bottom of the reactor which is usually a result of microbial growth and incoming sludge.

Seghezzo *et al.* (1998) reported that in a pilot plant UASB reactor, internal mixing was not optimal for sewage treatment (4–20°C) thus producing dead space and reduced process efficiency. In order to improve the process efficiency, an adequate influent distribution was sought. The use of effluent recirculation in combination with a taller reactor (a larger height to diameter ratio) resulted in the development of EGSB. Usually EBRs, as opposed to EGSB, have biofilm that is adsorbed onto support particles. An example of EBR is that of Tsuno *et al.* (1996) who employed them for the degradation of pentachlorophenol (PCP) anaerobically where the granular activated carbon was used as a support.

18.6 Biofilms employed to treat dairy industry wastewater

Application of biofilms in treating dairy industry wash water is another important area. In a recent report, dairy industry wash water was treated using a horizontal flow biofilm reactor system (Rodgers *et al.*, 2008). In this study, a horizontal flow biofilm reactor (HFBR) with step-feed was tested in the laboratory to remove organic carbon and nitrogen from an agricultural strength synthetic wash water. This study was conducted over a 212-day period and under steady state 99.7% carbon was removed. The nitrogen removal percentage was 98.3%. The authors reported that no clogging of media occurred during the study. The reactor system was simple to construct and operate.

18.7 Biofilms employed to treat oily sea water

Often there are oil leaks into sea or water streams that are toxic to marine life or may ultimately reach ground water. To avoid toxicity of such oil leaks, contaminated water or sea water should be treated to degrade toxic chemicals. In a number of studies, removal of oil from sea water has been reported (Al-Awadhi *et al.*, 2003; Radwan *et al.*, 2002). Al-Awadhi *et al.* (2003) established biofilms rich in hydrocarbon degrading bacteria. The biofilms were established on gravel particles and glass plates. The microbial consortia in the biofilms included filamentous cynobacteria, picoplankton,

and diatoms. Hydrocarbon utilizing bacteria *Acinetobactor calcoaceticus* and nocardioforms were, in part, attached to filaments of cyanobacteria. These studies were performed in batch cultures and it was demonstrated that the attached biofilms were able to remove (or perhaps utilize) crude oil from contaminated sea water. Radwan *et al.* (2002) employed a mineral medium containing crude oil as the sole carbon and energy source to identify 10 different macroalgae and bacteria capable of using oil. It was concluded that macroalgae found in Arabian Gulf sea water were coated with biofilms rich in oil utilizing/degrading bacteria. In another report bacterial biofilms were found associated with corroded oil pipelines (Neria-Gonzalez *et al.*, 2006).

18.8 Biofilms for production of industrial chemicals

18.8.1 Biofilms for ethanol production

Biofuels such as ethanol and butanol are large volume low value products. For this reason they should be produced economically using the most efficient reactor systems (Table 18.1). In order to increase the rate of ethanol

Table 18.1 A comparison of ethanol productivities in adsorbed cell biofilm reactors using various biofilm supports and cultures

System/Support	Reactor type	Culture	Productivity [$gL^{-1}\ h^{-1}$]	Reference
Biofilm reactors				
Resin	Packed bed	*Z. mobilis*	377.4 (P_{dv})	Krug and Daugulis, 1983
Vermiculite	Packed bed[d]	*Z. mobilis*	210 (P_{dv})	Bland *et al.*, 1982
Sugarcane bagasse[a]	Packed bed	*S. cerevisiae*	28.6[b]	Tyagi and Ghose, 1982
Polypropylene	Packed bed[e]	*Z. mobilis*	536[c]	Kunduru and Pometto, 1996
Plastic composite	Packed bed	*S. cerevisiae*	76[c]	Kunduru and Pometto, 1996
Batch/continuous suspended cell (control)				
Continuous	CSTR	*Z. mobilis*	5.0[b]	Kunduru and Pometto, 1996
		S. cerevisiae	5.0[b]	Kunduru and Pometto, 1996
Continuous	CSTR	*S. cerevisiae*	3.35[b]	Tyagi and Ghose, 1982

a – The support was reported as an adsorbent of natural origin (perhaps sugarcane bagasse); b – Not reported whether based on total reactor volume or void volume; c – Not reported (possibly based on void volume); d – Cone shaped reactor; e – Trickling packed bed reactor; P_{dv} – Productivity based on reactor void volume.

production Bland *et al.* (1982) employed a *Zymomonas mobilis* biofilm reactor to produce ethanol in an expanded bed bioreactor. The cells of *Z. mobilis* were adsorbed onto vermiculite and the culture formed an active biofilm. In this reactor, a productivity of 105 gL^{-1} h^{-1} (based on total volume) was obtained at a dilution rate of 3.6 h^{-1} while in a control batch free cell reactor a productivity of <4gL^{-1} h^{-1} was achieved. The enhanced (>25 times) productivity reported here is due to the formation of active biofilm onto the adsorbent.

In another report on ethanol production, adsorbed cells of *Saccharomyces cerevisiae* were used in a packed bed continuous bioreactor (Tyagi and Ghose, 1982; Table 18.1). The cells were immobilized onto a support of natural origin, possibly sugarcane bagasse and the amount of cells that was adsorbed was 0.13 gg^{-1} support. In this biofilm reactor, the authors reported a productivity of 28.6 gL^{-1} h^{-1} as compared to 3.35 gL^{-1} h^{-1} in a free cell continuous process. The dilution rates in the biofilm reactor and free cell continuous system were 0.47 h^{-1} and 0.65 h^{-1}, respectively. Although such biofilm reactors (also known as immobilized cell reactors) are typically operated at higher dilution rates than the free cell continuous reactors, it is not clear why the authors used a lower dilution rate in the biofilm reactor. It should be noted that product yield was also improved as compared to that achieved in a batch reactor.

Ion exchange resins are good supports for forming biofilms. Since resins have charge on them, bacterial cells can be adsorbed onto them thus forming uniform biofilm layers. This concept was employed by Krug and Daugulis (1983) to produce ethanol in high productivity reactors using *Z. mobilis*. These authors showed that a cationic macroreticular resin was the most efficient adsorbent to immobilize cells of *Z. mobilis*. The adsorbed (immobilized) cells were used in a continuous column and 100 gL^{-1} glucose solution was fed to the reactor. As a result of formation of biofilm, the reactor productivity was measured at 135.8 gL^{-1} h^{-1} (void volume based productivity, P_{dv} = 377.4 gL^{-1} h^{-1}). Unfortunately, the reactor stopped working due to excessive cell growth after a period of 200 h.

Kunduru and Pometto (1996) and Demirci *et al.* (1997) are among other authors that used biofilm reactors for ethanol production. Kunduru and Pometto (1996) studied ethanol production in continuous reactors using biofilm supports of polypropylene or plastic composite. Employing *Z. mobilis* and a bacterial support of polypropylene, an overwhelming high productivity of 536 gL^{-1} h^{-1} was obtained at a dilution rate of 15.36 h^{-1}. In a control free cell fermentation, a productivity of 5 gL^{-1} h^{-1} was obtained at a dilution rate of 0.5 h^{-1}. The direction of feed flow in the biofilm reactor was from top to bottom. Kunduru and Pometto (1996) used another biofilm reactor of *S. cerevisiae* (support material: plastic composite) and reported a productivity of 76 gL^{-1} h^{-1} at a dilution rate of 2.88 h^{-1}. In a control experiment a reactor productivity of 5 gL^{-1} h^{-1} was obtained at a dilution rate of 0.5 h^{-1}. Unlike the above biofilm reactor, this biofilm reactor was fed at the

bottom, and the product was obtained from the top. It is suggested that for an appropriate comparison, direction of liquid flow in both the reactors should have been the same.

In a report on enhancing biofilm formation, Demirci *et al.* (1997) developed a new support for the growth of *S. cerevisiae*. A mixture of ground soybean hulls (or oat hulls), complex nutrients, and polypropylene was extruded at high temperature into disks and rings. The purpose of including nutrients into the support was to encourage cell growth. However, it is possible that heat sensitive nutrients may have been inactivated during extrusion at high temperature. Also, polypropylene film may have covered the nutrients, thus making them unavailable to the culture for cell growth. Since no data have been provided on the time period of formation of biofilm or thickness of biofilm, it is difficult to compare this support with others.

In a more recent study, Qureshi *et al.* (2004a) produced ethanol in a biofilm reactor of genetically engineered *Escherichia coli* using xylose, a five carbon sugar as substrate. The biofilm was formed on clay brick particles and the bioreactor was operated in a continuous mode for 103 days. The reactor was operated at various dilution rates, and reactor productivity was found to be improved compared to a free cell batch process.

18.8.2 Butanol production in biofilm reactors

Butanol is a superior biofuel and has 30% more energy content than ethanol on a mass basis. It can be used in internal combustion engines and can be mixed with gasoline in any proportion. This chemical and biofuel can be produced from various substrates including cellulosic biomass (these cultures can utilize hexoses and pentoses), whey permeate, and starch using *Clostridium acetobutylicum* or *C. beijerinckii*. Butanol production has been investigated in batch, fed-batch, free cell continuous, immobilized (including biofilm) cell continuous, and cell recycle continuous reactors (Maddox, 1989).

Continuous immobilized cell and cell recycle reactors offer higher productivities than batch and free cell continuous reactors. In addition to achieving a high productivity, a major advantage of immobilized cell technology (and cell recycle) is that cell mass is not washed out at high flow rates.

An early report on adsorption (biofilm formation) of cells of *C. acetobutylicum* for the production of butanol was that of Forberg and Haggstrom (1985). They used beechwood shavings as an adsorption support for cells. Over a period of time, an active biofilm was formed on the wood shavings, and a reactor productivity as high as 1.53 $gL^{-1}\ h^{-1}$ was observed (compared to <0.1–0.38 $gL^{-1}\ h^{-1}$ in control batch fermentation). This work was followed by examining production of butanol in an adsorbed cell biofilm reactor of *C. acetobutylicum* P262 from whey permeate (Qureshi and Maddox, 1987). It should be noted that biofilm formation on this support

was rapid, and a reactor productivity of 4.5 gL^{-1} h^{-1} was achieved, which was superior to that obtained in any previously reported butanol production reactor system.

Following these reports, Welsh *et al.* (1987) investigated the use of a number of adsorption supports for butanol production by *C. acetobutylicum* in batch and continuous systems. The adsorbents that were used included coke, kaolinite, and gel white (a montmorillonite clay). Coke was found to be superior to the other supports for adsorption. A maximum concentration of acetone butanol ethanol (ABE) in the effluent of the reactor was reported to be 12 gL^{-1} at a dilution rate of 0.1 h^{-1}, thus resulting in a productivity of 1.2 gL^{-1} h^{-1}.

Following the above reports, an intensive study was performed on biofilm formation using *C. acetobutylicum* P262 onto numerous supports (Qureshi *et al.*, 2005). It has been observed that *C. acetobutylicum* and *C. beijerinckii* form visual biofilm layers in 2–4 days (in packed bed reactors), and reactors become productive after the 4th day of continuous operation. The techniques of adsorption and reactor operation have been reported elsewhere (Qureshi and Maddox, 1987; Qureshi *et al.*, 2000; Welsh *et al.*, 1987). It has been observed that not all the supports are suitable for adsorption. It has also been observed that during biofilm formation onto bonechar, the culture produces higher concentration of polysaccharide between days 2 and 4 (Qureshi *et al.*, 2005).

It is interesting that some supports accumulated greater cell concentrations (*C. acetobutylicum* P262) and were more solventogenic than the others (Qureshi *et al.*, 2005). At this stage, we are not aware what makes some supports better than others for biofilm formation and cell accumulation. From some supports it was easier to wash away the cells while from others, such as bonechar and clay brick, it was more difficult. During our studies on butanol production, it was observed that approximately 0.9–1.0 gL^{-1} cells were present in the effluent (Qureshi *et al.*, 1988; Qureshi *et al.*, 2004b) of the reactor. We have demonstrated that the cells that were present in the effluent of the reactor were those that grew on the surface of the support, rather than those that grew in liquid medium inside the reactor (Qureshi *et al.*, 2004b), suggesting that a tremendous amount of cell growth activity occurred on the surface of the biofilm in these *C. beijerinckii*/*C. acetobutylicum* cultures. The thickness of biofilm that is formed in *C. acetobutylicum* cultures can range from few cell layers to as many as 35 or more. Figure 18.1 shows adsorbed cells and biofilm formed by *C. acetobutylicum* P262 onto bonechar. Similar observations on biofilm formation were observed for *C. beijerinckii* (Qureshi *et al.*, 2000).

Butanol production in various types of reactor systems has been intensively studied (Maddox, 1989; Qureshi and Maddox, 1988; Ezeji *et al.*, 2005). The biofilm reactor systems for butanol production can be varied from vertical PBR, horizontal PBR, compartmentalized reactor, double series reactors, and FBR. The PBRs and FBRs are different in the sense that FBR

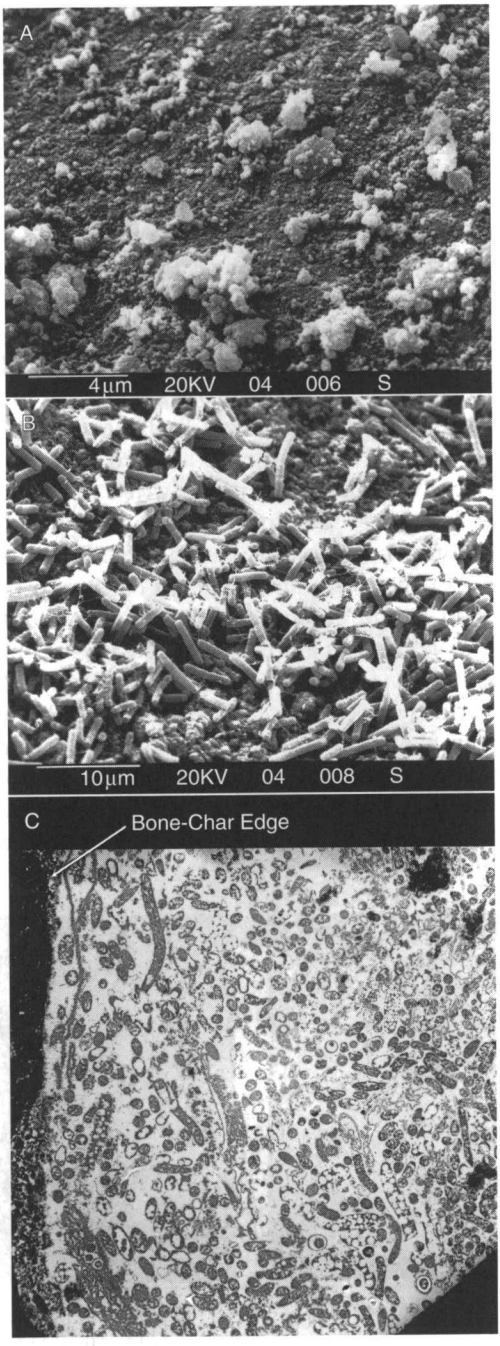

Fig. 18.1 Scanning electron micrograph of adsorbed cells of *C. acetobutylicum* P262 onto bonechar. a) bonechar (magnification 5500); b) adsorbed cells onto bonechar (magnification 2200); c) transmission electron micrograph of adsorbed cells (magnification 2300). Similar figures (1a, b, c) with different magnification were published previously in the following article: Qureshi N, Paterson A H J and Maddox I S (1988), 'Model for continuous production of solvents from whey permeate in a packed bed reactor using cells of *Clostridium acetobutylicum* immobilized by adsorption onto bonechar', *Appl Microbiol Biotechnol*, 29, 323–328. Reprinted 'with kind permission from Springer Science+Business Media' (see above article).

is started with support <10% of the reactor's volume while packed beds are filled up to 90% of their volume. PBRs often clog due to excessive cell growth while FBRs do not clog. In FBRs, the bed is fluidized either by recycling fermentation broth (mixture of fermentation medium containing cell mass, residual sugar and products), using anaerobic gases (N_2 or CO_2 and H_2 in case butanol fermentation) or air (for other aerobic systems). Cell growth occurs all around the support particles and over a period of time the volume of biofilm particles becomes many fold greater than the support particle (Fig. 18.2). It should be noted that in FBRs cell growth occurs on the particles in spite of the broth's high flow rates (Qureshi and Maddox, 1991). In this type of fluidized bed reactor liquid flow velocity ranges from 40 to 60 ms^{-1}. The reader is advised that despite such a high flow velocity, the culture maintains its growth as a biofilm. This biofilm reactor was used for the production of butanol from whey permeate in continuous operation for >4 months (unpublished results – Qureshi and Maddox). Newly adsorbed *C. acetobutylicum* cells onto bonechar grow in an exponential manner and accumulation of biomass continues with time. Figure 18.3 shows a picture of a fluidized bed reactor for butanol production from whey permeate using cells of *C. acetobutylicum* P262.

Among the various types of reactors used for butanol production, adsorbed cell biofilm reactors (cells adsorbed onto bonechar and clay brick) offered the highest reactor productivities ranging from 6.5 (Qureshi and Maddox, 1988) to 15.8 (Qureshi *et al.*, 2000) $gL^{-1}\ h^{-1}$ (as compared to 0.10–0.38 $gL^{-1}\ h^{-1}$ in batch reactors) (Table 18.2). Membrane cell reactors

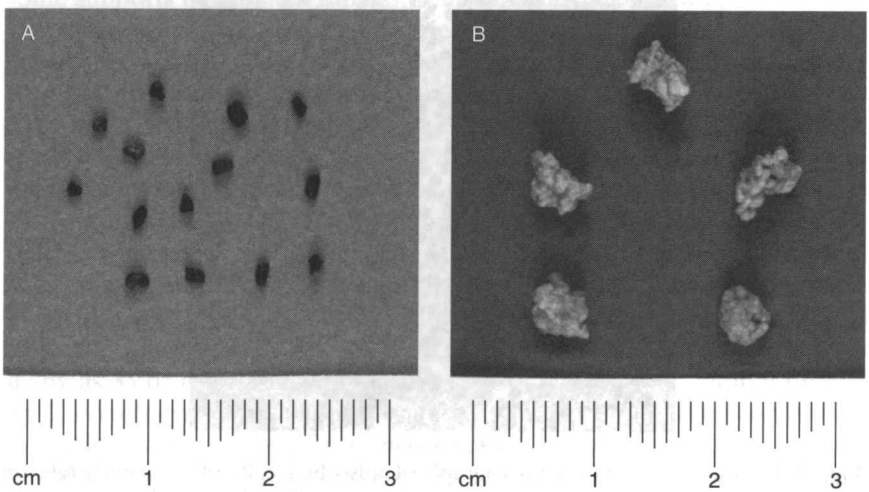

Fig. 18.2 A picture of biofilm particles of *C. acetobutylicum* P262 used in a fluidized bed reactor for butanol production from whey permeate (Adapted from Qureshi *et al.*, 2005; Open Access Journal). a) bonechar support particles before growth; b) bonechar particles covered after cell growth and formation of biofilm.

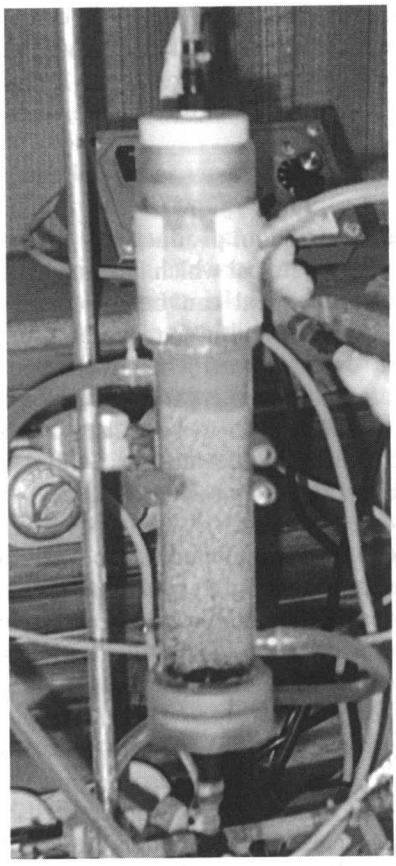

Fig. 18.3 A picture of a fluidized bed biofilm reactor used for butanol production from whey permeate (Adapted from Qureshi *et al.*, 2005; Open Access Journal).

Table 18.2 Production of solvents (acetone butanol ethanol; ABE) in packed bed biofilm reactors of *C. acetobutylicum/C. beijerinckii*

Culture/ support	Maximum solvent [gL^{-1}]	Maximum productivity [gL^{-1} h^{-1}]	Accumulated biomass [gL^{-1} reactor vol]	Reference
C. acetobutylicum				
Bonechar	9.3 (0.30)	6.50 (1.5)	74.0	Qureshi and Maddox, 1988
C. beijerinckii				
Clay brick	7.9 (2.00)	15.8 (2.00)	73.7	Qureshi *et al.*, 2000

Numbers in bracket are dilution rates (h^{-1}) at which solvent and productivity were obtained.

also offer high productivities (6.5 gL^{-1} h^{-1}) (Pierrot *et al.*, 1986; Afschar *et al.*, 1985); however, biofilm reactors have proved to be superior to these reactors. It should be noted that of the various supports tested, bonechar and clay brick were found to be most suitable, and strong biofilms were formed on these supports.

18.8.3 Biofilms in 2,3-butanediol production

2,3-butanediol is another chemical which has numerous applications in the chemical industry. This chemical can be produced from glucose, whey permeate, and hydrolyzed agricultural residues using biofilm reactors of *Klebsiella pneumonia* or *K. oxytoca*. In an attempt to improve reactor productivity in 2,3-butanediol fermentation, Maddox *et al.* (1988) adsorbed cells of *Klebsiella pneumoniae* on to bonechar in a similar fashion as *C. acetobutylicum* P262 (Qureshi and Maddox, 1987). During the 2,3-butanediol fermentation, a productivity of 11.7 gL^{-1} h^{-1} was obtained, which was the highest reported productivity for this system. Prior to this work Shazer and Speckman (1984) reported a productivity of 1.04 gL^{-1} h^{-1} in 2,3-butanediol fermentation using *Bacillus polymyxa* in a membrane cell reactor. This work clearly demonstrated that bonechar adsorbed cells of *K. pneumoniae* resulted in superior productivities. Table 18.3 compares 2,3-butanediol reactor productivity achieved in a biofilm reactor as compared to various other reactor types. It should be noted that although high reactor productivity was obtained in the adsorbed cell reactor, *Klebsiella pneumonia* cells did not adsorb onto bonechar surfaces as strongly as *C. acetobutylicum* P262. Rather, cells were entrapped in between bonechar particles. However, it is anticipated that there were significant amounts of cells sitting on the surface of bonechar as the bonechar surface area was large. At the end of fermentation, it was observed that unlike cells of *C. acetobutylicum*, *K. pneumoniae* cells were easily washed away. It is not known whether *K. pneumoniae* cells produce

Table 18.3 A comparison of 2,3-butanediol productivity in a packed bed biofilm reactor with productivities reported in other reactor systems

Reactor type	Culture	Substrate	Productivity [gL^{-1} h^{-1}]	Reference
Biofilm, continuous	*K. pneumoniae*	Whey permeate	11.70	Maddox *et al.*, 1988
Batch (control)	*A. aerogenes*	Glucose	1.10	Syblayrolles and Goma, 1984
Continuous reactor (free cells)	*K. pneumoniae*	Glucose	4.25	Ramachandran and Goma, 1987

polysaccharide which can facilitate adsorption of cells to the adsorbent's surface. Even though *K. pneumoniae* cells do not form firm layers of cells, these reactors were still highly productive.

18.8.4 Production of acetic acid or vinegar using biofilms

Acetic acid or vinegar has numerous applications in food, feed, and the chemical industry and can be produced employing a biological process. Acetic acid production in trickling bed biofilm reactors is a mature technology and is exercised at the commercial scale (Table 18.4) (Crueger and Crueger, 1989). In addition to the use of trickling bed biofilm reactors, a submerged process was also developed in the late 1940s. Acetic acid is produced by one of the bacteria grouped in the two genera, *Gluconobacter* and *Acetobacter*. The species that are used commercially include *Acetobacter aceti*, *A. pasteurianus*, and *Gluconobacter oxydans*. In the trickling bed biofilm reactor (volume 60 m^3), beechwood shavings are packed and the starting material (alcohol solution) is sprayed over the surface. Initially, nutrients and bacteria are added to this solution to promote growth and biofilm formation on the beechwood shavings. The liquid trickles to the bottom of the reactor containing acetic acid. In order to increase concentration of acetic acid, the liquid is cooled and recycled back to the top of the reactor. Of the alcohol added, approximately 90% is converted to acetic acid during the trickling process. Approximately 120 gL^{-1} acetic acid is obtained in 72 h, thus resulting in a productivity of 1.67 gL^{-1} h^{-1}.

Table 18.4 Production of various other chemicals in biofilm reactors (Adapted from Qureshi *et al.*, 2005)

Product/reactor type	Adsorption support	Productivity [gL^{-1} h^{-1}]	Reference
Acetic acid			
Trickling bed biofilm reactor	Beechwood shavings	1.67 (120)	Crueger and Crueger, 1989
Lactic acid			
Agitating continuous reactor	Fibrous bed (cloth)	2.5 (126)	Tay and Yang, 2002
Fumaric acid			
Rotary continuous reactor	Plastic discs	4.25 (85)	Cao *et al.*, 1996
Strirred-tank (control)	None	0.91	Cao *et al.*, 1996
Succinic acid			
Repeated batch fermentations	Plastic discs	–	Urbance *et al.*, 2003

Numbers in bracket – product concentration in gL^{-1}.

18.8.5 Production of lactic acid employing biofilms

Production of lactic acid in biofilm reactors is another example of industrial chemical production using such reactors. Demirci *et al.* (1993a) evaluated a number of supports for biofilm formation using lactic acid producing cultures. It has been reported that the best biofilms were obtained with *Pseudomonas fragi*, *Streptomyces viridosporus*, and *Thermoactinomyces vulgaris* when used in combination with polypropylene composite chips. The polypropylene composite chips were obtained by extruding polypropylene and 25% (w/w) agricultural material to the desired size and shape. Subsequently, a number of reports appeared from the same group on synthesizing, evaluating, and using various supports for biofilm formation and lactic acid production (Demirci *et al.*, 1993b; Ho *et al.*, 1997a, b, c). In one of the reports (Ho *et al.*, 1997a), lactic acid was produced in repeated batch cultures using a biofilm reactor. The reactor productivity was improved from 2.78 to 4.26 gL^{-1} h^{-1}. A maximum lactic acid concentration of 60 gL^{-1} was produced in biofilm reactors where a plastic composite support was used for cell adsorption.

In a study on the production of lactic acid by adsorbed cells of *Rhizopus oryzae*, the culture was immobilized on a fibrous-bed and used in a bioreactor (Tay and Yang, 2002). In this reactor (fed-batch), a productivity of 2.5 gL^{-1} h^{-1} was obtained with a high yield of 90% and a high product concentration of 127 gL^{-1}. When glucose (substrate) was replaced with cornstarch, yield improved to 100% and productivity decreased to 1.65 gL^{-1} h^{-1}. Using starch as a substrate, a product concentration of 126 gL^{-1} was achieved.

Other reports on using cell support for cell growth and lactic acid production are those of Park *et al.* (1998) and Sun *et al.* (1999). Park *et al.* (1998) used 3 gL^{-1} mineral support (Aid-Plus; ML-50D, Mizusawa Chemical Co., Niigata, Japan) and 5 ppm polyethylene oxide to flocculate the culture and change mycelial morphology from a large pellet to mycelial flocs. Sun *et al.* (1999) immobilized cells of *R. oryzae* in polyurethane foam cubes. In a recent report on production of lactic acid using lactic acid bacteria (LAB), it was reported that the investigated bacteria (*Lactobacillus plantarum*, *L. brevis*, and *L. fructivorans*) adhered, accumulated, and formed biofilms on glass cover slips (Kubota *et al.*, 2008).

18.8.6 Biofilms for fumaric acid production

Biofilm reactors have also been used successfully for the production of fumaric acid (Cao *et al.*, 1996) and mineral ore treatment (Crueger and Crueger, 1989). In a study, Cao *et al.* (1996) used plastic discs to adsorb cells of *Rhizopus oryzae* to produce fumaric acid from glucose. The use of the biofilm reactor resulted in an increase in reactor productivity from 0.9 gL^{-1} h^{-1} (in a free cell stirred-tank reactor) to 4.25 gL^{-1} h^{-1} in the biofilm reactor. In the latter reactor, fumaric acid concentration up to 85 gL^{-1} was

obtained from 100 gL^{-1} glucose. The fermentation time was shorter and took 20 h as compared to 72 h in the free cell reactor.

18.8.7 Biofilms for succinic acid production

The industrial potential of succinic acid fermentation was recognized as early as the late 1970s (Zeikus *et al.*, 1999). Succinic acid ($HOOCCH_2CH_2COOH$) is a dicarboxylic acid, and can be used as a feedstock chemical for the production of high value products including 1,4-butanediol, tetrahydrofuran, adipic acid, γ-butyrolactone, and n-methylpyrrolidone (Zeikus *et al.*, 1999) for numerous applications in agriculture, food, medicine, plastics, cosmetics, and textiles. Succinic acid can be produced in biofilm reactors. In a recent study on succinic acid production using *Actinobacillus succinogenes*, Urbance *et al.* (2003) employed customized plastic composite support (PCS) (Cotton *et al.*, 2001) and 20 other different PCS blends. The customized plastic composite support (PCS) blends were screened for biofilm formation and succinic acid production. These customized PCS blends demonstrated 70% yields for succinic acid production as compared to 64% in suspended cell bioreactor using *A. succinogenes* (Urbance *et al.*, 2003). Table 18.4 shows production of various industrial chemicals (in particular acids) in biofilm reactors.

18.9 Length of operation of biofilm reactors

While producing chemicals in biofilm reactors, it should be one of the objectives to operate the reactor for a long period of time, thus enhancing the economics of the process. Packed bed biofilm reactors often clog due to excessive cell growth. It should be noted that reactor blockage depends on a number of factors including cell growth rate, packing density of the support, and supply of nutrients. Packed bed biofilm reactors have been typically operated ranging from 2 weeks to 4 months. Tyagi and Ghose (1982) operated a packed bed biofilm reactor of *S. cerevisiae* for a period of 35 days, while Qureshi *et al.* (2004a) used a packed bed biofilm reactor of *E. coli* for a period of 103 days for ethanol production in continuous operation. However, it was observed that packed bed biofilm reactors of *C. acetobutylicum* P262/*C. beijerinckii* blocked sooner than 103 days due to excessive cell growth of these cultures. In order to prolong life of the reactor, feed media that limit cell growth (perhaps deficient in nutrients) and still produce chemicals at a rapid rate should be attempted as successfully applied by Qureshi and Maddox (1987) and Qureshi *et al.* (2004a). In addition to the reactor blockage due to excessive growth, influent to the reactor plays an important role in prolonging the life of the reactor. Reactor feed containing suspended and particulate solids, in particular wastewater influents, may

Table 18.5 Length of operation of various biofilm reactors used for the production of industrial chemicals (Adapted from Qureshi *et al.*, 2005)

Chemical produced	Reactor type	Length of operation [days]	Dilution rate [h^{-1}] (productivity gL^{-1} h^{-1})	Reference
Butanol	Packed bed	61 days	0.30–1.00 (0.98–4.10)	Qureshi and Maddox, 1987
	Fluidized bed	>4 months	0.33–1.37 (1.65–5.10)	Unpublished data[1]
Lactic acid	Various reactors	30 days	– –	Tay and Yang, 2002
Ethanol	Packed bed	35 days	0.12–0.48 (7.80–28.60)	Tyagi and Ghose, 1982
	Packed bed	103 days	0.04–0.12 (1.10–2.58)	Qureshi *et al.*, 2004a
	Packed bed	60 days	0.50–5.76 (5.00–74.88)	Kunduru and Pometto, 1996

[1] Qureshi and Maddox.
– Not reported.

also clog the reactor. Table 18.5 shows the lengths of operation of various biofilm bioreactors.

18.10 Industrial/pilot-plant level biofilm reactors

Biofilm reactors have successfully been used for wastewater treatment (Lettinga *et al.*, 1980; 1983; Nicolella *et al.*, 2000; Seghezzo *et al.*, 1998; Tsuno *et al.*, 1996; Iza, 1991; Schmidt *et al.*, 2004) (Fig. 18.4). In these industrial biofilm reactors, cell mass concentration as high as 30–40 gL^{-1} could be maintained (Nicolella *et al.*, 2000 [Fig. 18.4]; Shieh and Keenan, 1986). As a result of superior efficiency, biofilm reactors are being used throughout the world with a number of full-scale applications for industrial chemical production and wastewater treatment. Examples of these reactors operating in The Netherlands and Brazil have been reported elsewhere (Nicolella *et al.*, 2000; reviewed in Qureshi *et al.*, 2005). Similarly, commercial production of acetic acid or vinegar using biofilm reactors has been exercised for many years. Production of these chemicals has been reported by Crueger and Crueger (1989). Large biofilm fermentors up to 60 000 L have been used. Often beechwood shavings are used as a support for biofilm formation. For this system, trickling bed reactors have been used with an exit product concentration up to 120 gL^{-1} and a productivity of 1.67 gL^{-1} h^{-1}.

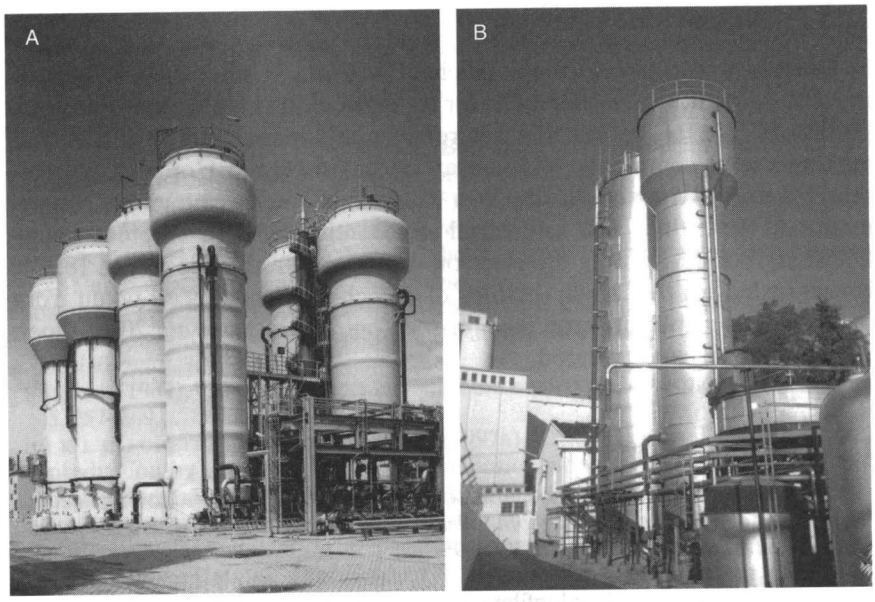

Fig. 18.4 Full-scale biofilm reactors: (a) biothane biofilm airlift suspension and expanded granular sludge blanket (Biobed) reactors at Gist Brocades, Delft (The Netherlands); (b) Pagues CIRCOX (foreground; 140 m^3) and internal circulation (background; 385 m^3) reactors at a brewery in Brazil. Reprinted from 'Nicolella C, van Loosdrecht M C M, Heijnen S J (2000), "Particle-based biofilm reactor technology", *Trends in Biotechnology*, 18, 312–320', with permission from Copyright Clearance Center's Rightslink service. Elsevier has partnered with Rightslink to license its content online.

18.11 Future trends and conclusions

A comparison of biofilm reactors with other reactor systems suggests that biofilm reactors are simple, offer higher productivity, and biofilm supports are economically available. In biofilm reactors, cells can be adsorbed within the reactor without the use of any chemicals, and the reactors can be operated for prolonged periods, thus helping to reduce the process cost. Some examples of their use at a large scale have been given in the above section. It is clear that use of biofilm reactors (in laboratory scale) has been consistently increasing for the production of various industrial chemicals. As productivity in these simple biofilm reactors is high, their full potential should be employed for biotechnological/biological conversion processes. Biofilm reactors have potential to be operated for long periods (Table 18.5) thus offering economic benefits over other reactor types. For further reading on biofilms and their formation, the reader is referred to the comprehensive articles published by Costerton *et al.* (1987) and O'Toole *et al.* (2000).

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